Journal of Chromatography A 1652 (2021) 462344

Contents lists available at ScienceDirect

Journal of Chromatography A

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

A selective and efficient microfluidic method-based liquid phase microextraction for the determination of sulfonamides in urine samples

Samira Dowlatshah[a,b], Elia Santigosa[c], Mohammad Saraji[b], María Ramos Payán[a,*]

[a] Department of Analytical Chemistry, Faculty of Chemistry, University of Seville, c/Prof. García González s/n, 41012, Seville, Spain
[b] Department of Chemistry, Isfahan University of Technology, Isfahan 84156–83111, Iran
[c] Department of Analytical Chemistry, Universitat Autónoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

ARTICLE INFO

Article history:
Received 22 February 2021
Revised 7 June 2021
Accepted 8 June 2021
Available online 15 June 2021

Keywords:
Microfluidic device
Liquid phase microextraction
High performance liquid chromatography
Supported liquid membrane
Sulfonamides

ABSTRACT

Liquid phase microextraction (LPME) into a microfluidic has undergone great advances focused on down-scaled and miniaturized devices. In this work, a microfluidic device was developed for the extraction of sulfonamides in order to accelerate the mass transfer and passive diffusion of the analytes from the donor phase to the acceptor phase. The subsequent analysis was carried out by high performance liquid chromatography with UV-DAD (HPLC-DAD). Several parameters affecting the extraction efficiency of the method such as the supported liquid membrane, composition of donor and acceptor phase and flow rate were investigated and optimized. Tributyl phosphate was found to be a good supported liquid membrane which confers not only great affinity for analytes but also long-term stability, allowing more than 20 consecutive extractions without carry over effect. Under optimum conditions, extraction efficiencies were over 96 % for all sulfonamides after 10 minutes extraction and only 10 µL of sample was required. Relative standard deviation was between 3-5 % for all compounds. Method detection limits were 45, 57, 54 and 33 ng mL^{-1} for sulfadiazine (SDI), sulfamerazine (SMR), sulfamethazine (SMT) and sulfamethoxazole (SMX), respectively. Quantitation limits were 0.15, 0.19, 0.18 and 0.11 µg mL^{-1} for SDI, SMR, SMT SMX, respectively. The proposed microfluidic device was successfully applied for the determination of sulfonamides in urine samples with extraction efficiencies within the range of 86-106 %. The proposed method improves the procedures proposed to date for the determination of sulfonamides in terms of efficiency, reduction of the sample volume and extraction time.

Introduction

Sulfonamides are an important group of bacteriostatic agents that receive great interest due to its use to prevent infections, treat diseases and to promote growth [1]. Some authors consider that sulfonamides are implicated in the increasing prevalence of antibiotic resistance in humans [2–5] and its excessive use in veterinary medicine generates a public health problem. This means that selective and sensitive methodologies are required to control and monitor the presence of these compounds in our environment. To date, different instrumental techniques have been used for the analysis of sulfonamides using different detection systems as for example thin layer chromatography [6], amperometric detection [7], high performance liquid chromatography (HPLC) [8–12], capillary electrophoresis (CE) [13–15] and gas chromatography (GC) and gas chromatography – mass spectrometry (GC-MS) [16]. However, most procedures have previously required solid phase extraction (SPME) procedures in one or more stages [17,18]. In the last decade, liquid phase microextraction (LPME) procedures have been widely used due to the advantages they present, such as high pre-concentration and excellent clean-up. In this line, methods-based ion pair [19], two and three phase hollow fiber liquid phase microextraction (HF-LPME) [20–23], dispersive liquid-liquid microextraction (DLLME) [24], voltage assisted liquid phase microextraction (VA-LPME) [25], single drop liquid phase microextraction

* Corresponding author.
E-mail address: ramospayan@us.es (M.R. Payán).

https://doi.org/10.1016/j.chroma.2021.462344

(SD-LPME) [26] and capsule phase microextraction [27] were reported for the determination of sulfonamides. Achieving high extraction efficiency is still challenging in these methods in which a long analysis time [19–23, 28], high organic solvent amount and high sample volume consumption are frequently required. In most cases, the extractions are carried out using a solid support that acts as a membrane separating two phases. An organic solvent is deposited on this membrane as the supported liquid membrane (SLM). The selection of the SLM is one of the critical parameters to achieve good extraction efficiency. Among the methods mentioned, some are based on an SLM. . For example 1-octanol [20,22,23], 2-octanone [25] and an ionic liquid (IL) and tri-n-octyphosphine oxide [21] were previously selected as optimal SLM. These methods required between 30 and 480 minutes of extraction and 4-50 mL of sample volume, offering good enrichments between 14-1000. The amount of solvent used was of the order of milliliters and a new liquid membrane was necessary between each extraction, without being reusable, consequently increasing the amount of organic solvent in the case of carrying out repetitive measurements.

In this line, and over the recent decade, sample preparation based on microfluidic systems have attracted considerable attention not only due to the ability to decrease extraction time and costs but also because of the capability of reduction or elimination of reagent consumption. These miniaturized sample systems have shown great potential for extracting drugs of different nature, as well as in biological and environmental applications [29–41]. LPME has also been implemented into microfluidic systems. In this way, the analytes are extracted from a donor phase to an acceptor phase through a supported liquid membrane (SLM) by passive diffusion. Among the materials available for the manufacture of the device, polymethyl methacrylate is the one that has offered the best advantages and most versatility to date, as well as low cost [29]. The working dimensions of these devices have proven to be a good alternative to improve mass transfer between both phases. The miniaturization of these channels has also reduced the volume of sample and reagents required, especially organic solvent. Furthermore, this contributes to decrease extraction times, which are often relatively long in traditional systems. Therefore, the development of a miniaturized method for the determination of sulfonamides could significantly improve the extraction efficiency by accelerating the mass transfer through the SLM.

The main objective of this work is to develop an efficient, selective and environmental-friendly microfluidic method based liquid phase microextraction to significantly increase the extraction efficiency of sulfonamides, reducing the extraction time and the required sample volume, offering excellent clean up, and improving previously reported procedures.

2. Experimental

2.1. Chemicals and materials

All chemicals were of analytical-reagent grade. Sulfadiazine (SDI), Sulfamerazine (SMR), Sulfamethazine (SMT) and Sulfamethoxazole (SMZ) were provided from Fluka-Sigma-Aldrich (Madrid, Spain). Formic acid, sodium hydroxide, chloric acid, 2-nitrophenyl octyl ether (NPOE), dihexyl ether (DHE), and 1-octanol were purchased from Fluka-Sigma-Aldrich (Madrid, Spain). Methanol, acetonitrile, nonanol, decanol, undecanol, and tributyl phosphate (TBP) were supplied from Merck (Darmstadt, Germany). The stock solutions of the sulfonamides were prepared in methanol (100 mg L^{-1}) and preserved at 4°C in a refrigerator. Working solutions were daily provided by dilution of the stock solutions with deionized water (from a Milli-Q Plus water purification system (Millipore, Billerica, MA, USA)). A micro-syringe pump (Cetoni GmbH, Korbussen, Germany) was utilized to introduce the liquid phases into the microchip device.

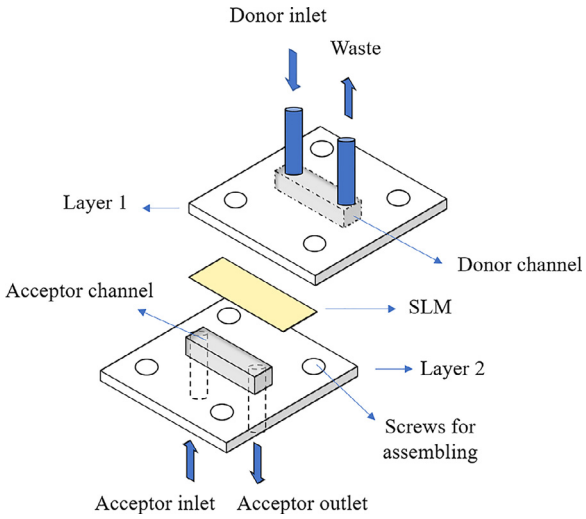

Fig. 1. Scheme of the microfluidic device based LPME.

2.2. Chromatographic conditions

The chromatographic equipment to carry out the separation of the compounds consisted of an Agilent 1100 series liquid chromatograph (Barcelona, Spain) equipped with a G1312A Bipump systems, diode array detector (DAD) and an autosampler G1313A as injector. Separations were carried out at 25 °C using a LiChroCART 75-4 Purosphere STAR RP-18e 3mm (75 mm x 4.0 mm i.d.) (VWR, Germany) 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 acetonitrile (component B) at a flow rate of 0.8 mL min^{-1}. A gradient program was used from 85 % A to 70 % A in 10 minutes for the separation. The injection volume was 7 µL. Additionally, 3 min were waited between injections to achieve the reequilibration of the column to the initial conditions. The wavelengths used for DAD were 254 nm for all analytes. The chromatogram was completed in 10 min and the retention times were 2.5, 3.3, 4.19 and 8.29 for SDI, SMR, SMT and SMX, respectively.

2.3. Chip device fabrication and procedure

The microfluidic device consisted of two poly(methyl methacrylate) (PMMA) plates assembled through four screws and a laser ablation cutter (Epilog Mini 24–30 W) was used for its fabrication at the following conditions: writing speed of 40 %, power of 24 %, a resolution of 1500, and a frequency of 5000. Fig. 1 shows a scheme of the microfluidic device proposed. Each layer contains a channel (13 mm length, 70 μm deep and 3mm wide) and a flat membrane is placed covering the entire channel separating the donor and acceptor channel from each other. The membrane is impregnated with 3 µL of organic solvent (TBP) and subsequently closed using four screws. The device can be reused, opened and closed as many times as necessary. Each channel has two holes: one for inlet solution and another for outlet solution. Both, the donor phase (containing the analytes) and the acceptor phase are introduced into the microfluidic device for the microextraction by using a microsyringe pump (Cetoni GmbH, Korbussen, Germany), which operate at 1 µL min^{-1}. First, 5 min were waited for SLM stabilization and later, the acceptor phase was collected during 10 minutes in a micro insert tube and then injected into the HPLC system for analysis.

2.4. Calculations of extraction efficiency

Extraction efficiency for each analyte was calculated according to the following equation for each analyte (eq 1):

$$EE\ (\%) = \frac{C_{f,a,outlet}}{C_{i,s,inlet}}\ x\ \frac{v_a}{v_s}\ x\ 100 \qquad \text{(Eq. 1)}$$

where $C_{f,a,outlet}$ is the final concentration of the analyte at the outlet of the acceptor channel, $C_{i,s,inlet}$ is the initial concentration of the analyte in the sample, and, v_a *and* v_s, are the acceptor and sample flow rate, respectively.

2.6. Real samples

Urine samples were analyzed using the microchip device to evaluate its applicability. Both samples were adjusted to pH 4.0 with HCl solution and spiked at three different levels (0.5, 1 and 3 µg mL^{-1}). All samples were filtered through Pall NylafloTM nylon membrane filter 0.45 µm (Pall Corporation, Ann Arbor, Michigan, USA). Each sample was directly extracted by the microfluidic LPME device.

3. Results and discussion

3.1. Supported liquid membrane selection

Previous studies carried out on LPME-based microfluidics have described optimal geometric characteristics for passive diffusion and good mass transfer [29, 35, 36]. Based on that, an initial device of 13 mm length, 70 μm deep and 3mm wide was designed for the optimization of the experimental parameters. Sulfonamides have two dissociation constants related to pK_{a1} and pK_{a2}. PK_{a1} and pK_{a2} corresponds to a basic amino group (-NH_2) and an acid group (-NH-SO_2-), respectively. The amino group is capable of gaining a proton while the amide of the acid group is capable of releasing a proton under specific pH conditions. Within the pH range between the first and second pK_a, the molecule is predominantly neutral, while at pH above its second pK_a value, the molecule is negatively charged. The pK_{a1} -pK_{a2} values are 1.6 - 6.5, 1.58-6.90, 2.07-7.49 and 1.85-5.60 for SDI, SMR, SMT, SMZ respectively [20].

Supported liquid membrane was the first experimental parameter to optimize since it is a critical parameter that is directly related to the nature of the analytes. Solvent selection was based on the following requirements: water immiscibility, non-volatility, affinity towards analytes, and compatibility with PMMA plates. Based on these requirements and previously reported solvents compatible with sulfonamides and LPME into a chip [20,29,35,36], 2-nitrophenyl octyl ether (NPOE), dihexyl ether, nonanol, decanol, undecanol, and tributyl phosphate were tested as organic solvent. Donor phase solution, acceptor phase solution and flow rate were fixed at pH 2.5 (HCl), pH 12 (NaOH) and 1 μL min^{-1}, respectively, for the study. At those conditions, sulfonamides are found in their neutral form and in their ionized form in the donor and acceptor phase, respectively. The microfluidic device was cleaned with miliQ water and a new sheet membrane was used for each different organic solvent. Table 1 shows the highest extraction efficiency for all analytes when using TBP as SLM. Four replicate experiments were carried out to test the repeatability and a relative standard deviation (RSDs %) below 7 % was obtained for all analytes, except when using NPOE (RSD% 9-12). Thus, tributyl phosphate was selected for further experiments.

3.2. Donor and acceptor solutions optimization

Donor phase pH was studied within the pH range of 2-6 to ensure the analytes to be in their neutral form for passive diffusion, while the acceptor phase composition was fixed at pH 12 (NaOH) and the flow rate at 1 μL min^{-1}. As seen in Fig. 2, the highest peak area was observed at pH 4 for all analytes while no significant difference was observed between the rest of the pHs studied. In the next step, a range of pH between 9.0-12 was tested to study the acceptor phase composition while the donor pH was adjusted at 4 for all experiments. Based on Fig. 3, the peak area increased up to pH 12 and best results were obtained at the mentioned pH for all sulfonamides. A study of the long-term stability of sulfonamides was carried out at optimal pH's, especially at basic pH to ensure that sulfonamides were not degraded at pH 12. The relative standard deviation based on four replicate experiments was below 6 %. Therefore, a pH 4 and pH 12 were adjusted throughout the rest of experiments as donor and acceptor composition, respectively.

3.3. Donor phase flow rate optimization

Table 1
Extraction efficiencies (RSD %) of the sulfonamides using different organic solvents as SLM.

Extraction efficiency % (RSD%, n=4)				
	SDI	SMR	SMT	SMZ
Decanol	7 (1)	11 (1)	13 (1)	53 (1)
Undecanol	5 (1)	8 (1)	10 (2)	43 (1)
Nonanol	8 (5)	18 (5)	14 (5)	45 (6)
Octanol	21 (1)	42 (1)	32 (2)	78 (2)
DHE	5 (2)	7 (6)	10 (4)	46 (4)
NPOE	13 (12)	17 (10)	21 (9)	47 (10)
TBP	74 (6)	76 (6)	80 (5)	81 (4)

a Sample: pH 2.5 containing the four compounds each at 1 µg mL^{-1}, acceptor phase: pH 12, sample and acceptor flow rate: 1 µL min^{-1}, extraction time: 10 min.

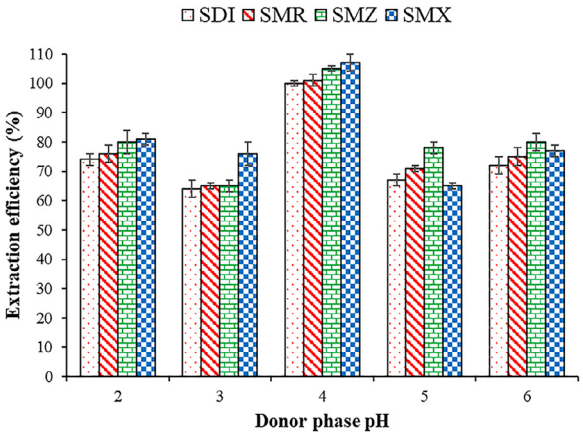

Fig. 2. Optimization of the donor phase composition. SLM: TBP. Acceptor phase pH 12. Flow rate: 1µL min^{-1} (acceptor and donor phase).

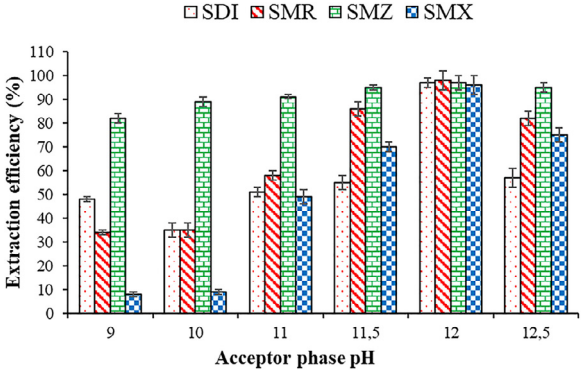

Fig. 3. Optimization of the acceptor phase composition. SLM: TBP. Donor phase pH 4. Flow rate: 1µL min^{-1} (acceptor and donor phase).

Table 2
Calibration parameters using standard solution in water, method detection limit (MLOD), method quantitation limit (MLOQ) and extraction efficiencies at optimal conditions.

	MLOD (μg mL^{-1})	MLOQ (μg mL^{-1})	Regresion Equation	R^2	EE *
SDI	0.045	0.15	Y=12519x-987.65	0.9997	101 (3)
SMR	0.057	0.19	Y= 14358x-1432.7	0.9996	96 (5)
SMT	0.054	0.18	Y= 9787.5x-126.56	0.9998	98 (2)
SMX	0.033	0.11	Y= 12836x+340.52	0.9996	98 (3)

* % Extraction efficiency (%RSD, n=4) in water

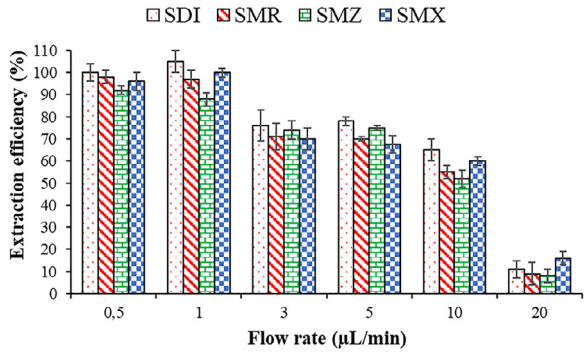

Fig. 4. Donor flow rate optimization. SLM: TBP. Donor phase pH 4. Acceptor phase pH 12.

There are two flows when working with microfluidics in sample pretreatment: donor and acceptor. Both flows are decisive in the extraction efficiency of the procedure. However, previous studies have shown that the extraction efficiency would significantly decrease with greater acceptor flow since the time that this phase is in contact with the donor phase (containing the analytes) will decrease [31, 36, 38]. For this reason, the acceptor flow has been set to 1 μL min^{-1} avoiding loss of extraction efficiency. In some cases, depending on the analyte and how fast the mass transfer is, the extraction efficiency does not decrease drastically at low flows and its study is interesting since sometimes there is an enrichment phenomenon with acceptable efficiencies [36]. Then, donor flow rate influence was evaluated within the range of 0.5-20 μL min^{-1} using an acceptor flow rate of 1 μL min^{-1}. As seen in Fig. 4, highest extraction efficiencies were observed at 0.5 or 1 μL min^{-1} and no significant difference was observed between both flow rate. Efficiencies over 96 % was observed for all analytes. The residence time of the sample decreased when the flow rate increased so, consequently, a decrease in extraction efficiency was observed at higher donor flow rate. Therefore, a flow rate of 1 μL min^{-1} is selected in order to achieve a faster extraction. The efficiencies obtained once the procedure is optimized were between 96-100 %, so the selected geometry at the beginning has been successful and no further optimization is needed since the device already has miniaturized characteristics of short (13 mm) and shallow (0.07 mm) channels.

The carry over effect was tested by analyzing individual extractions with a new membrane and after consecutive extractions, without observing memory effects.

4. Analytical performance

Microfluidic method based LPME was evaluated for the determination of four sulfonamides by fixing the experimental parameters at optimal conditions as described above. A calibration curve was constructed using a least-square linear regression analysis at seven different concentrations from 0.15 to 10 μg mL^{-1}, 0.19 to 10 μg mL^{-1}, 0.18 to 10 μg mL^{-1} and 0.11 to 10 μg mL^{-1} for SDI, SMR, SMT and SMX, respectively. A linear relationship with r^2 values over 0.9996 was obtained in all cases. Table 2 shows the calibration parameters of the method: detection limits (LODs, S/N=3), quantitation limits (LOQs, S/N=10) and extraction efficiencies for all analytes. LODs between 0.033-0.057 μg mL^{-1} were obtained for all sulfonamides. Three concentration levels of the calibration curve (0.28, 1 and 5 μg mL^{-1}) were selected to test the repeatability (n=4) and intraday repeatability (n=4, 15 days), obtaining a relative standard deviation between 3- 6 % and below 3-5 % for repeatability and intraday repeatability for all compounds, respectively. Calibration curve was prepared with standard solutions of the analytes in water. Extraction efficiencies between 96-102 % were obtained for all analytes. Finally, different microfluidic devices with the same geometry were used to test the reproducibility. Each device was tested replacing the membrane three times and a relative standard deviation below 6 % was obtained for all analytes.

5. Comparison with other setups

The performance of this chip was compared with previous methodologies for sulfonamides extraction, in terms of extraction time, extraction efficiency, relative recovery and sample solution volume (Table 3). As seen, the authors express the results based on extraction efficiency (EE %), enrichment factor (EF) and / or relative recovery (RR %). The extraction efficiency is defined as the percentage of the mole numbers of the analyte extracted into the acceptor phase respect to the moles number of the analyte originally present in the donor solution and which also depends on the volume of each phase. On the other hand, the enrichment factor is defined as the ratio of the analyte concentration in the analyte-containing acceptor to the initial concentration of analytes in the donor solution and the relative recovery by the percentage of the amount of analyte recovered in the acceptor solution from spiked real samples. Different methods for the extraction of sulfonamides have been reported with high enrichment factors between 121-996 [22], 58-135 [21], 268-664 [19] and 200-1000 [20]. Moreover, the sample volume required is relatively high, with a minimum sample volume between 4000-8000 μL [21,22] up to 50000 μL [20]. The extraction time of these methods ranges from 20 min [19] to 8 h [21], a significantly long time. Furthermore, some of them require more than one sample pretreatment stage prior to analysis [19,21]. Other methods have been reported with shorter extraction times between 20-30 min [18, 24, 26–28, 40] and extraction efficiencies between 70-77 % [28], 50-60 % [18], 56-100 % [42] and 12-18 % [27]. Some of these procedures also require more than one treatment stage prior to analysis, lengthening the total analysis time [19, 27, 28] and those that only consist of one extraction stage require at least 1000 μL of sample volume. One of the most relevant advantages when using flat membrane microfluidic systems is that in many cases, the supported liquid membrane is reusable and therefore allows consecutive extractions to be carried out without the need to change the membrane or add extracting solvent. As seen in Table 3, the presented microfluidic method provides the highest extraction efficient for all sulfonamides in real sample (human urine). In addition, the required sample volume was de-

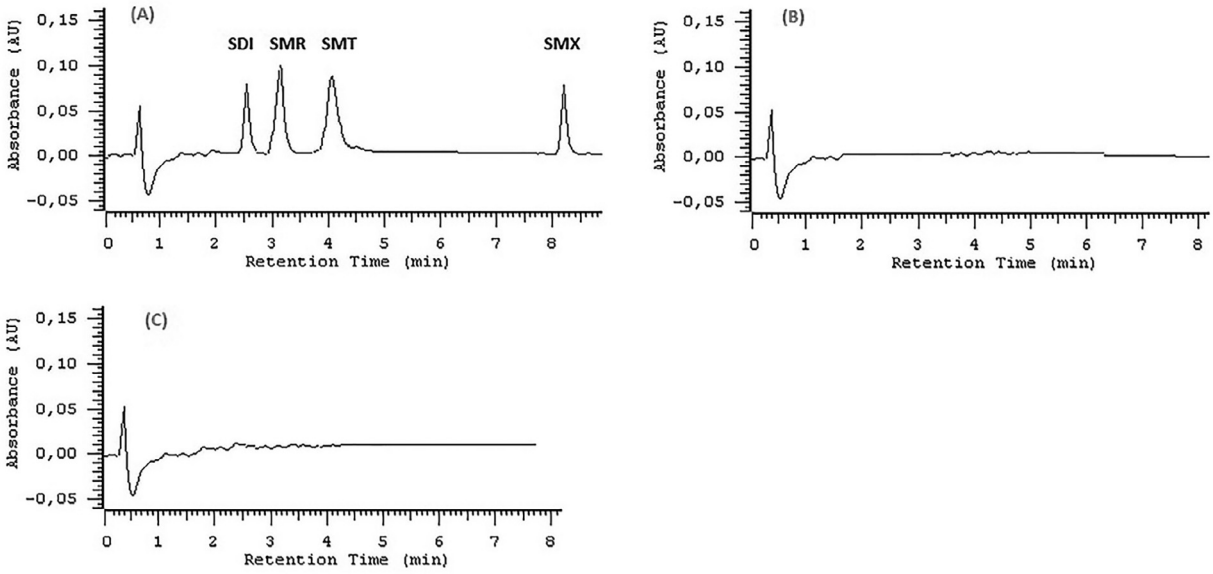

Fig. 5. Chromatogram of a (A) spiked human urine at 1 μg mL^{-1} and (B) blank human urine and (C) blank urine chromatogram of a reused membrane.

Table 3
Comparison of μLPME procedure with other analytical methods for extraction of sulfonamides.

Technique (*Analysis*)	Real sample	Extraction Time (min)	Sample volume (μl)	Enrichment factor	% Extraction Efficiency/ (*R:recovery from spiked real samples)	Consecutive extractions	Ref.
HF-LPME-HPLC/UV	water	360	50000	200-1000	-/*(R: 32-100)	No	[20]
SUPRASs-solvent based LPME- HPLC/UV&	Plasma serum	20	200	6.1-6.7	70-77 /*(R: 85-90)	No	[28]
Ion pair Emulsification LPME-HPLC-UV/Vis	water	20	10000	268-664	41-97/*(R: 104)	No	[19]
Capsule phase microextraction-HPLC/UV	milk	30	2000	-	12-18	No	[27]
SD-LPME-HPLC/UV	water	20	1500	-	-/*(R: 63-115)	No	[26]
DLLME-HPLC/UV	water	10	5000	-	-/*(R: 78-117)	No	[24]
SPME-LC/MS	meat	40	15000	-	4-27	No	[17]
HF-LPME-HPLC/UV	water	480	4000	58-135	-/*(R: 82-103)	No	[21]
HF- LPME-CE/ED	water	60	8000	121-996	-/*(R: 75-109)	No	[22]
VA-LPME-HPLC/DAD	water	30	4000	-	-/*(R: 78-98)	No	[25]
IT-SPME-HPLC/UV	milk	25	>1000	-	50-60/*(R: 11-97)	No	[18]
IL magnetic bar-HF-LPME/FLD	water	25	6000	-	56-100/*(R: 19-94)	No	[42]
HF-LPME-UPLC/FLD	water	60	8000	14-60	-/*(R: 56-113)	No	[23]
μLPME-HPLC/UV	Urine	10	10	-	87-103/(R: 84-100)	YES	This work

HF-LPME: Hollow fiber-liquid phase microextraction, SD-LPME: Single drop liquid phase microextraction, DLLME: Dispersive liquid liquid microextraction, SPME: Solid phase microextraction, VA-LPME: Voltage-Assisted Liquid-Phase Microextraction, IT-SPME: In-tube solid-phase microextraction, IL magnetic bar-HF-LPME: ionic liquid magneticc bar based hollow fiber liquid phase microextraction, FLD: Fluorescence detection, ED: electrochemical detection.

Table 4
Extraction efficiencies (average of three determinations ± standard deviation) from 1 μg mL^{-1} spiked urine samples.

Samples	Spiked level (μg mL^{-1})	SDI	SMR	SMT	SMZ
Urine (non-diluted)	0.5	97.6 ± 3.9	86.2 ± 4.0	88.1 ± 1.6	91.8 ± 3.2
	1	95.3 ± 2.2	86.8 ± 3.3	89.3 ± 2.4	92.4 ± 1.8
	3	106.1 ± 5.3	87.0 ± 2.0	86.8 ± 1.9	84.8 ± 2.6

creased between 20 and 5000 times compared to existing methods and it also required shorter extraction times (only 10 min).

6. Real samples analysis

The applicability of the microfluidic device proposed was investigated in human urine samples. Urine samples were collected from a 35 year-old healthy adult female volunteer and undiluted samples were spiked at three different concentration levels (0.5, 1 and 3 μg mL^{-1}) of SDI, SMR, SMT and SMZ. Experiments were analyzed in triplicate for each of the concentrations. All samples were submitted to the microfluidic device using the optimal experimental conditions, and the extract collected was analyzed by HPLC-DAD. Similar extraction efficiencies were obtained for each analyte regardless of concentration. Extraction efficiencies between

95-106 %, 85-88 %, 83-90 % and 83-92 % for SDI, SMR, SMT and SMZ were obtained, respectively. As seen in Table 4, the relative standard deviation after triplicate experiments was below 5.5 % for all analytes and the membrane was stable for more than 20 consecutive extractions using urine samples with no carry over effect. Fig. 5 shows the corresponding DAD chromatogram from spiked human urine (A), a blank (B) using two different membranes. Fig. 5C shows a blank chromatogram after washing the membrane previously used for consecutive extractions.s.

4. Conclusion

This work presents for the first time an efficient microfluidic method for the determination of sulfonamides and its successfully application in urine samples. The presented microfluidic system significantly improves in terms of sample and reagent volume and analysis time, offering high extraction efficiencies compared to previous reported methodologies. The method was also successfully applied in urine sample with extraction efficiencies between 83 and 106 % for all sulfonamides with only a urine sample volume consumption of 10 µL after 10 minutes extraction time and excellent clean-up. TPB has been demonstrated to be a good organic solvent as extractant which significantly contributes to the stability of the microfluidic system when the method is applied to consecutive urine extractions, thus reducing the cost of instrumentation.

Author Statements

Samira Dowlatshah: Formal Analysis, Investigation. Elia Santigosa: Data curation, Mohammed Saraji: writing original draft, 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.

Acknowledgements

This work was partially supported by Microliquid S.L. in the frame of the bilateral collaborative project XploreChip P01158. Samira Dowlatshah thanks the Ministry of Science, Research and Technology of the Islamic Republic of Iran and the Research Council of Isfahan University of Technology (IUT) for the scholarship.

References

[1] K. Dost, D.C. Jones, G. Davidson, Determination of sulfonamides by packed column supercritical fluid chromatography with atmospheric pressure chemical ionisation mass spectrometric detection, Analyst 125 (2000) 1243–1247.
[2] H.C. Wegener, The Consequences for Food Safety of the Use of Fluoroquinolones in Food Animals, N. Engl. J. Med. 340 (1999) 1581–1582.
[3] D. Ferber, Science 288 (2000) 792–794.
[4] P.D. Fey, T.J. Safranek, M.E. Rupp, E.F. Dunne, E. Ribot, P.C. Iwen, P.A. Bradford, F.J. Angulo, S.H. Hinrichs, Ceftriaxone-Resistant Salmonella Infection Acquired by a Child from Cattle, N. Engl. J. Med. 342 (2000) 1242–1249.
[5] J. Krungkrai, S.R. Krungkrai, C.T. Supuran, Carbonic anhydrase inhibitors: Inhibition of Plasmodium falciparum carbonic anhydrase with aromatic/heterocyclic sulfonamides-in vitro and in vivo studies, Bioorganic Med. Chem. Lett. 18 (2008) 5466–5471.
[6] S. Babić, D. Ašperger, D. Mutavdžić, A.J.M. Horvat, M. Kaštelan-Macan, Determination of sulfonamides and trimethoprim in spiked water samples by solid-phase extraction and thin-layer chromatography, J. Planar Chromatogr. - Mod. TLC. 18 (2005).
[7] C. Reguera, M.C. Ortiz, A. Herrero, L.A. Sarabia, Optimization of a FIA system with amperometric detection by means of a desirability function. Determination of sulfadiazine, sulfamethazine and sulfamerazine in milk, Talanta 75 (2008) 274–283.
[8] D. Teshima, B. Hino, Y. Itoh, R. Oishi, Simple and simultaneous determination of sulphapyridine and acetylsulphapyridine in human serum by column-switching high-performance liquid chromatography, J. Clin. Pharm. Ther. 27 (2002) 403–408.
[9] J. Tuerk, M. Reinders, D. Dreyer, T.K. Kiffmeyer, K.G. Schmidt, H.M. Kuss, Analysis of antibiotics in urine and wipe samples from environmental and biological monitoring - Comparison of HPLC with UV-, single MS- and tandem MS-detection, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 831 (2006) 72–80.
[10] A. Kaufmann, P. Butcher, K. Maden, M. Widmer, Ultra-performance liquid chromatography coupled to time of flight mass spectrometry (UPLC-TOF): A novel tool for multiresidue screening of veterinary drugs in urine, Anal. Chim. Acta. 586 (2007) 13–21.
[11] D.C.G. Bedor, T.M. Gonçalves, M.L.L. Ferreira, C.E.M. de Sousa, A.L. Menezes, E.J. Oliveira, D.P. de Santana, Simultaneous determination of sulfamethoxazole and trimethoprim in biological fluids for high-throughput analysis: Comparison of HPLC with ultraviolet and tandem mass spectrometric detection, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 863 (2008) 46–54.
[12] J.K. Johannessen, I. Christiansen, D.R. Schmidt, E. Petersen, S.H. Hansen, Simultaneous determination of pyrimethamine, sulfadiazine and N-acetyl-sulfadiazine in plasma for monitoring infants in treatment of congenital toxoplasmosis, J. Pharm. Biomed. Anal. 36 (2005) 1093–1098.
[13] C.L. Ng, H.K. Lee, S.F.Y. Li, Determination of sulphonamides in pharmaceuticals by capillary electrophoresis, J. Chromatogr. A. 632 (1993) 165–170.
[14] R.B. Hoff, F. Barreto, T.B.L. Kist, Use of capillary electrophoresis with laser-induced fluorescence detection to screen and liquid chromatography-tandem mass spectrometry to confirm sulfonamide residues: Validation according to European Union 2002/657/EC, J. Chromatogr. A. 1216 (2009) 8254–8261.
[15] Q. xun Dang, Z. pei Sun, D.K. Ling, Separation of sulphonamides and determination of the active ingredients in tablets by micellar electrokinetic capillary chromatography, J. Chromatogr. A. 603 (1992) 259–266.
[16] K.J. Bisceglia, J.T. Yu, M. Coelhan, E.J. Bouwer, A.L. Roberts, Trace determination of pharmaceuticals and other wastewater-derived micropollutants by solid phase extraction and gas chromatography/mass spectrometry, J. Chromatogr. A. 1217 (2010) 558–564.
[17] K.H. Lu, C.Y. Chen, M.R. Lee, Trace determination of sulfonamides residues in meat with a combination of solid-phase microextraction and liquid chromatography-mass spectrometry, Talanta 72 (2007) 1082–1087.
[18] Y. Wen, M. Zhang, Q. Zhao, Y.Q. Feng, Monitoring of five sulfonamide antibacterial residues in milk by in-tube solid-phase microextraction coupled to high-performance liquid chromatography, J. Agric. Food Chem. 53 (2005) 8468–8473.
[19] B. Ebrahimpour, Y. Yamini, M. Rezazadeh, A sensitive emulsification liquid phase microextraction coupled with on-line phase separation followed by HPLC for trace determination of sulfonamides in water samples, Environ. Monit. Assess. 187 (2015) 1–13.
[20] M. Ramos Payán, M.A. Bello López, R. Fernández-Torres, M.V. Navarro, M.C. Mochón, Hollow fiber-based liquid phase microextraction (HF-LPME) for a highly sensitive HPLC determination of sulfonamides and their main metabolites, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 879 (2011) 197–204.
[21] Y. Tao, J.F. Liu, X.L. Hu, H.C. Li, T. Wang, G.Bin Jiang, Hollow fiber supported ionic liquid membrane microextraction for determination of sulfonamides in environmental water samples by high-performance liquid chromatography, J. Chromatogr. A. 1216 (2009) 6259–6266.
[22] F. Tong, Y. Zhang, F. Chen, Y. Li, G. Ma, Y. Chen, K. Liu, J. Dong, J. Ye, Q. Chu, Hollow-fiber liquid-phase microextraction combined with capillary electrophoresis for trace analysis of sulfonamide compounds, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 942–943 (2013) 134–140.
[23] L. Yang, Y. Shi, J. Li, T. Luan, In situ derivatization and hollow-fiber liquid-phase microextraction to determine sulfonamides in water using UHPLC with fluorescence detection, J. Sep. Sci. 41 (2018) 1651–1662.
[24] A.V. Herrera-Herrera, J. Hernández-Borges, T.M. Borges-Miquel, M. ángel Rodríguez-Delgado, Dispersive liquid-liquid microextraction combined with ultra-high performance liquid chromatography for the simultaneous determination of 25 sulfonamide and quinolone antibiotics in water samples, J. Pharm. Biomed. Anal. 75 (2013) 130–137.
[25] M.H. Wang, H.W. Chang, S.P. Wang, Analysis of Sulfonamides by Liquid Chromatography Mass Spectrometry and Capillary Electrophoresis Combing with Voltage-Assisted Liquid-Phase Microextraction, J. Chinese Chem. Soc. 60 (2013) 1479–1483.
[26] X. Guo, D. Yin, J. Peng, X. Hu, Ionic liquid-based single-drop liquid-phase microextraction combined with high-performance liquid chromatography for the determination of sulfonamides in environmental water, J. Sep. Sci. 35 (2012) 452–458.
[27] D. Georgiadis, A. Tsalbouris, A. Kabir, K.G. Furton, V. Samanidou, Novel capsule phase microextraction in combination with high performance liquid chromatography with diode array detection for rapid monitoring of sulfonamide drugs in milk, J. Sep. Sci. 42 (2019) 1440–1450.
[28] P. Bogdanova, A. Pochivalov, C. Vakh, A. Bulatov, Supramolecular solvents formation in aqueous solutions containing primary amine and monoterpenoid compound: Liquid phase microextraction of sulfonamides, Talanta 216 (2020) 120992.
[29] M. Ramos Payán, Liquid - Phase microextraction and electromembrane extraction in millifluidic devices:A tutorial, Anal. Chim. Acta. 1080 (2019) 12–21.
[30] N.J. Petersen, H. Jensen, S.H. Hansen, S.T. Foss, D. Snakenborg, S.Pedersen Bjergaard, On-chip electro membrane extraction, Microfluid. Nanofluidics. 9 (2010) 881–888.

[31] B. Li, N.J. Petersen, M. Ramos Payán, S.H. Hansen, S.Pedersen Bjergaard, Design and implementation of an automated liquid-phase microextraction-chip system coupled on-line with high performance liquid chromatography, Talanta 120 (2014) 224–229.
[32] E. Santigosa Murillo, X. Muñoz Berbel, S. Maspoch, M. Muñoz, M. Ramos Payán, Impedance model for voltage optimization of parabens extraction in an electromembrane millifluidic device, J. Chromatogr. A. 1625 (2020) 461270.
[33] Y.A. Asl, Y. Yamini, S. Seidi, A novel approach to the consecutive extraction of drugs with different properties via on chip electromembrane extraction, Analyst 141 (2016) 311–318.
[34] M. Karami, Y. Yamini, Y. Abdossalami Asl, M. Rezazadeh, On-chip pulsed electromembrane extraction as a new concept for analysis of biological fluids in a small device, J. Chromatogr. A. 1527 (2017) 1–9.
[35] M. Ramos Payán, S. Maspoch, A. Llobera, A simple and fast Double-Flow microfluidic device based liquid-phase microextraction (DF-µLPME) for the determination of parabens in water samples, Talanta 165 (2017) 496–501.
[36] M. Ramos Payán, S. Maspoch, A. Llobera, An effective microfluidic based liquid-phase microextraction device (μLPME) for extraction of non-steroidal anti-inflammatory drugs from biological and environmental samples, Anal. Chim. Acta. 946 (2016) 56–63.
[37] N.J. Petersen, S.T. Foss, H. Jensen, S.H. Hansen, C. Skonberg, D. Snakenborg, J.P. Kutter, S. Pedersen-Bjergaard, On-Chip Electro Membrane Extraction with Online Ultraviolet and Mass Spectrometric Detection, Anal. Chem. 83 (2011) 44–51.
[38] M. Ramos Payán, H. Jensen, N.J. Petersen, S.H. Hansen, S.Pedersen Bjergaard, Liquid-phase microextraction in a microfluidic-chip – High enrichment and sample clean-up from small sample volumes based on three-phase extraction, Anal. Chim. Acta. 735 (2012) 46–53.
[39] Y.A. Asl, Y. Yamini, S. Seidi, M. Rezazadeh, Simultaneous extraction of acidic and basic drugs via on-chip electromembrane extraction, Anal. Chim. Acta. 937 (2016) 61–68.
[40] M. Ramos Payán, E. Santigosa, R. Fernández Torres, M.Á.Bello López, A New Microchip Design. A Versatile Combination of Electromembrane Extraction and Liquid-Phase Microextraction in a Single Chip Device, Anal. Chem. 90 (2018) 10417–10424.
[41] F. Zarghampour, Y. Yamini, M. Baharfar, M. Faraji, Simultaneous extraction of acidic and basic drugs via on-chip electromembrane extraction using a single–compartment microfluidic device, Analyst 144 (2019) 1159–1166.
[42] L. Yang, Y. Shi, J. Li, T. Luan, In situ derivatization and hollow-fiber liquid-phase microextraction to determine sulfonamides in water using UHPLC with fluorescence detection, J. Sep. Sci. 41 (2018) 1651–1662.